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5-Caffeoylquinic acid and caffeic acid orally administered suppress P-selectin expression on mouse platelets

Jae B. Park*

Diet, Genomics, and Immunology Laboratory, BHNRC, ARS, USDA, Beltsville, MD 20705, USA

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Abstract

Caffeic acid and 5-caffeoylquinic acid are naturally occurring phenolic acid and its quinic acid ester found in plants. In this article, potential effects of 5-caffeoylquinic acid and caffeic acid on P-selectin expression were investigated due to its significant involvement in platelet activation. First, the effects of 5-caffeoylquinic acid and caffeic acid on cyclooxygenase (COX) enzymes were determined due to their profound involvement in regulating P-selectin expression on platelets. At the concentration of 0.05 μM, 5-caffeoylquinic acid and caffeic acid were both able to inhibit COX-I enzyme activity by 60% ($P<.013$) and 57% ($P<.017$), respectively. At the same concentration, 5-caffeoylquinic acid and caffeic acid were also able to inhibit COX-II enzyme activity by 59% ($P<.012$) and 56% ($P<.015$), respectively. As expected, 5-caffeoylquinic acid and caffeic acid were correspondingly able to inhibit P-selectin expression on the platelets by 33% ($P<.011$) and 35% ($P<.018$), at the concentration of 0.05 μM. In animal studies, 5-caffeoylquinic acid and caffeic acid orally administered to mice were detected as intact forms in the plasma. Also, P-selectin expression was respectively reduced by 21% ($P<.016$) and 44% ($P<.019$) in the plasma samples from mice orally administered 5-caffeoylquinic acid (400 μg per 30 g body weight) and caffeic acid (50 μg per 30 g body weight). These data suggest that both 5-caffeoylquinic acid and caffeic acid orally administered can be absorbed and suppress P-selectin expression on mouse platelets.
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Keywords: 5-Caffeoylquinic acid; Chlorogenic acid; Caffeic acid; COX inhibitor; P-selectin; Platelet activation; Mice

1. Introduction

Caffeic acid is a phenylpropenoic acid and 5-caffeoylquinic acid is a caffeic acid ester, also known as a chlorogenic acid. They are commonly found in numerous plants including fruits, vegetables and coffee [1–4]. In plants, 5-caffeoylquinic acid is produced via forming an ester bond between the carboxyl group of caffeic acid and the 5-hydroxyl group of quinic acid [3]. 5-Caffeoylquinic acid and caffeic acid have been reported to decrease the risk of chronic diseases such as inflammation, cardiovascular disease and cancer [4,5]. Also, several studies suggested beneficial effects of fruits, vegetables and coffee consumption on cardiovascular and other diseases [5–8]. However, the effects of 5-caffeoylquinic acid and caffeic acid on cardiovascular diseases and their underlying mechanism have not been fully elucidated.

P-selectin is a 140-kDa, type 1 transmembrane glycoprotein commonly used as a biomarker for platelet activation. P-selectin is involved in platelet–leukocyte interactions and platelet–endothelium interactions via binding to P-selectin ligand 1 (PSGL-1) on leukocytes and endothelium [9–14]. Those interactions are often implicated in pathophysiological progress of several cardiovascular diseases such as atherosclerosis, angina, acute myocardial infarction and ischemic cerebral stroke [15–18]. P-selectin expression is mainly regulated by cyclooxygenase (COX) enzymes, catalyzing the conversion of arachidonic acid to prostaglandin H2, which is the intermediate molecule for prostacyclin and thromboxane A2. Currently, little is known about the effects of 5-caffeoylquinic acid and caffeic acid on P-selectin expression on platelets. Therefore, potential effects of 5-caffeoylquinic acid and caffeic acid on COX enzymes and P-selectin expression were investigated in this study,

* Tel.: +1 301 504 8365; fax: +1 301 504 9062.
E-mail address: jae.park@ars.usda.gov.

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using in vitro and in vivo models. Also, plasma concentrations of 5-caffeoylquinic acid and caffeic acid orally administered to mice were measured by high-performance liquid chromatography (HPLC) in order to validate the potential effects of 5-caffeoylquinic acid and caffeic acid on P-selectin expression in vivo.

2. Materials and methods

2.1. Materials

COX-I and COX-II enzymes, 5-caffeoylquinic acid, caffeic acid and other chemicals were purchased from Sigma Chemical (St. Louis, MO, USA).

2.2. COX inhibition assay

COX-I and COX-II activities were measured in a 96-well plate using a chemiluminescent COX kit (Assay Designs, Ann Arbor, MI, USA). Briefly, 50 μl of Tris-phenol buffer (100 μM Tris, 0.5 μM phenol buffer, pH=7.3) was added into the wells; 50 μl of hematin solution (hematin was dissolved in DMSO at 0.380 mg/ml, and diluted 5000-fold with 100 mM phosphate buffer, pH=7.5) and 50 μl COX-I (700 U) or COX-II (700 U) were added into the wells. The samples were incubated at room temperature for 5 min. After the incubation, 5-caffeoylquinic acid, caffeic acid or COX inhibitors were added. For an additional 10 min, the samples were incubated at room temperature (in the dark). Following the incubation, COX activity was measured using a luminometer, by injecting 50 μl of chemiluminescent COX substrate (4°C) and arachidonic acid, respectively. Relative light units output was measured to determine COX activity.

2.3. Measurement of P-selectin expression

Blood was collected in siliconized microfuge tubes containing 15% EDTA. The modified Tyrodes buffer [134 mM NaCl, 0.34 mM Na_2HPO_4, 2.9 mM KCl, 12 mM $NaHCO_3$, 20 mM HEPES, 5 mM glucose and 0.35% (w/v) bovine serum albumin, pH 7.0] was added to bring the sample volume to 100 μl. From the diluted samples, aliquots were placed in 12×75 polypropylene tubes along with the appropriate antibody and the modified Tyrodes buffer in a final volume of 200 μl. 5-Caffeoylquinic acid and caffeic acid were dissolved in ethanol and added to diluted blood samples, where the final ethanol volume never exceeded 0.5% (v/v) in both control and test tubes. Samples were analyzed for P-selectin (CD62p) expression on platelets within 1 h of the collection.

2.4. Determination of 5-caffeoylquinic acid and caffeic acid in plasma

5-Caffeoylquinic acid and caffeic acid were determined by HPLC after extraction from the blood plasma. To extract 5-caffeoylquinic acid and caffeic acid from plasma samples, the plasma samples (60 μl) were precipitated with methanol (40 μl) and centrifuged at 14,000×*g* for 10 min. The supernatant was injected onto an HPLC column. Spherisorb ODS2 (octadecyl silica 2; 5 μm, 4.6×250 mm) was used as the stationary phase to analyze 5-caffeoylquinic acid and caffeic acid in plasma samples, and an isocratic buffer of 50 mM NaH_2PO_4 (pH 4.3) containing 20% methanol was used as the mobile phase for the HPLC analyses. Peaks were detected by an electrochemical detector with four electrode channels (CoulArray, ESA, Chelmsford, MA, USA) and quantified by its software (v.1.0). For optimal measurement of 5-caffeoylquinic acid; the four channels were set at 100, 300, 550 and 800 mV, and they were quantitatively determined by an external standard method. Both 5-caffeoylquinic acid and caffeic acid could be reliably measured up to 0.01 μM, with linear detector response up to 10 μM.

2.5. Animal study

Swiss Webster mice 4-6 weeks old were purchased from Charles River (Wilmington, MA). Mice were placed in standard cages and housed in the environmentally controlled Beltsville Human Nutrition Research Center Animal Facility. The animal room was maintained at 20°C and 55% relative humidity. On arrival, mice were fed AIN-76A purified diet that provides the recommended amount of all nutrients required for maintaining optimal health. After 8 weeks, mice were assigned and remained to 3 groups (n=5) for 10 weeks. Mice in the first group (control) were orally administered distilled water (100 μl) using a dosing needle; mice in the second group were orally administered distilled water (100 μl) containing 5-caffeoylquinic acid (400 μg) and mice in the third group were orally administered distilled water (100 μl) containing caffeic acid (50 μg). Blood was collected via tail bleeding technique after the oral administrations, and blood samples from each group were used for P-selectin assay.

2.6. Statistical analysis

Treatments effects on the parameters measured were compared by analyzing the means for differences using either analysis of variance (ANOVA) or ANOVA by ranks, as appropriate. Differences were considered to be significant when $P<.05$. Data points represent the mean±S.D. of three or more samples.

3. Results

3.1. Effects of 5-caffeoylquinic acid and caffeic acid on COX-I enzyme

COX-I enzyme is constitutively expressed in numerous cells including platelets, and the COX-I enzyme is involved in prostaglandin homeostasis. The inhibition of COX-I enzyme is known to inhibit platelet activation via several mechanisms including the inhibition of P-selectin expression. COX-I inhibitors such as ibuprofen and